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Endothelial Progenitor Cells: More Than an Inflammatory Response?

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Abstract—The formation of new capillaries (angiogenesis) may be of clinical importance in facilitating reperfusion and regeneration of hibernating cardiac tissue after myocardial infarction and in microvascular ischemia. Evidence is accumulating that as part of the response to hypoxia, bone marrow-derived circulating endothelial progenitor cells (CEPs) are mobilized and subsequently differentiate into proper endothelial cells. There are also indications that such CEPs can facilitate endothelial repair and angiogenesis in vivo. It is not clear yet, however, whether these CEPs are essential for these adaptive processes or what the relative contribution of CEP is compared with that of other mononuclear inflammatory cells that are mobilized to areas of ischemia. Moreover, there are still many uncertainties about how cardiovascular risk factors alter CEP function. Particularly when therapeutically mobilizing CEPs, a further understanding of this issue is essential to assess the risk of potentially harmful side effects of altered CEP function. *(Arterioscler Thromb Vasc Biol. 2004;24:834-838.)*

Key Words: angiogenesis ■ atherosclerosis ■ endothelial progenitor

O ver the past 7 years, the discovery of the phenomenon that mononuclear cells in peripheral blood have the potential to differentiate into endothelial cells ex vivo as well as in vivo has opened up a new field of cardiovascular research.[1,2] It thus appears that such endothelial progenitor cells (EPCs) can be used therapeutically to restore damaged endothelium.[3,4] They can also incorporate into the endothelial monolayer and stimulate proliferation of neighboring endothelial cells, thus inducing the formation of new blood vessels.[5,6] Although the biology is not really understood, several pilot studies have suggested beneficial effects of infusion of mononuclear cells after myocardial infarction in animal models[7–10] and in humans.[11] The aim of the current review is to discuss some of the potential regulatory mechanisms involved in this phenomenon. In particular, we address the question of whether these progenitor cells are a specific subpopulation with stem cell properties or whether these cells merely reflect plasticity of the normal inflammatory response that occurs in occlusive vascular disease. Ultimately, understanding this biology will be a critical success factor for bringing progenitor cell therapy into the clinical arena.

Vascular Occlusion Leads to Inflammation

In the adult, vessels grow either via capillary sprouting (angiogenesis) or via remodeling of pre-existing arteriolar connections into collateral vessels (arteriogenesis).[12,13] Both processes occur on occlusion of a vessel, thus improving blood delivery and local perfusion of ischemic tissue. The regulation of the cellular processes involved in arteriogenesis

has recently been reviewed in this journal.[13] In this review, we focus on the contribution of bone marrow-derived progenitor cells in the (microcirculatory) angiogenic response to ischemia, a process referred to as (postnatal) vasculogenesis. The endothelial cell is key in initiating vasculogenesis. First, on vascular occlusion, endothelial cells will sense altered shear stress. Recent studies have shown that at low shear stress or oscillatory shear stress, endothelial cells typically will increase the expression of pro-oxidant enzymes, such as NADH oxidase,[14] and reduce the expression of anti-oxidant enzymes such as manganese superoxide dismutase,[15] thioredoxin reductase, and glutathione reductase.[16] As a result, altered shear stress during vascular occlusion will result locally in increased redox signaling.[17] One of the activated transcription factors is NF-κB, which plays a central role in the inflammatory response.[18] NF-κB activation in the endothelium results in the expression of adhesion molecules and the release of chemotactic factors for inflammatory cells.[19] In agreement, in situ nuclear translocation of NF-κB has been found in the vessel wall near regions of disturbed blood flow, like bifurcations, curvatures, and branching points.[20] The second parameter that endothelial cells sense during vascular occlusion is hypoxia. Basically, the endothelial cell is equipped with 2 systems to sense such hypoxia. One is the transcription factor HIF-1α.[21] Under normoxic conditions, this transcription factor will be hydroxylated on a conserved prolyl residue in a reaction with molecular oxygen. The hydroxylated prolyl group allows the Von Hippel-Lindau protein to bind and polyubiquinate the molecule, thereby
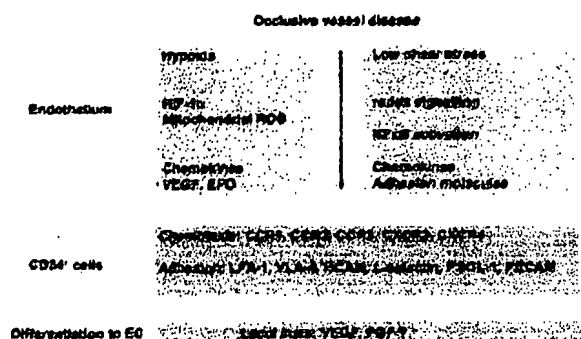
Figure 1. In atherosclerotic vascular disease, the endothelial cells will be exposed to hypoxia and altered shear stress. Both mechanisms induce redox signaling in the endothelial cell, with subsequent release of chemokines and surface expression of leukocyte adhesion molecules. The CD34^{+} hematopoietic stem cell is equipped with several receptors, which allow for chemotaxis, and with ligands for adhesion. Local cues such as the concentration of VEGF and basic FGF probably determine differentiation into endothelial-like cells.

earmarking HIF-1α for destruction by the proteasome. However, in hypoxia, this hydroxyprolination is reduced, allowing HIF-1α to accumulate and translocate to the nucleus to activate transcription of genes such as VEGF, EPO, and inducible NOS. The other oxygen-sensing mechanism in the endothelial cell is located in the mitochondria where, under normoxic conditions, oxidative phosphorylation occurs.[22] This reaction is constitutively inhibited by nitric oxide that is also produced in the mitochondria. When hypoxia occurs, nitric oxide starts to dominate and reduces electron transport toward its final acceptor, complex IV.[23] As a result, intermediate radical products such as hydrogen peroxide and reactive nitrogen species are produced by the mitochondria, again leading to redox signaling and subsequent activation of the endothelial cell. Taken together, altered shear stress and hypoxia will result in redox-mediated endothelial cell activation and release of chemokines, cytokines, and growth factors. Thus, occlusive vascular disease inevitably results in a local inflammatory response of the endothelium (Figure 1).

Endothelial Progenitor Cells: Part of the Inflammatory Response?

For some time, it has been suggested that as a consequence of such an inflammatory response, the ensuing recruitment of monocytes are instrumental not only in inducing collateral formation but also in promoting angiogenesis.[13,24] It is believed that paracrine release of cytokines and growth factors with known angiogenic properties, such as bFGF and TNFα, mediate these effects of monocytes on capillary sprouting.[25]

More recently, attention has also been drawn to other vasculogenic cell populations present in the mononuclear fraction of peripheral blood that may also be recruited to the activated endothelium in response to an ischemic insult. These are referred to as pluripotent stem cell or progenitor cell populations and include the CD34^{+} hematopoietic stem cells and subpopulations of CD34^{-} mononuclear cells, and even subpopulations of the peripheral blood monocytes. Isolation of each of these subpopulations and subsequent

culture in vitro could give rise not only to classical circulating blood cells such as monocytes/macrophages but also to unexpected phenotypes such as endothelial cells and myocytes.[1,26-28] Hence, the concept has evolved that vascular progenitors are recruited from the bone marrow to sites of tissue revascularization, where they participate in a paracrine way and also directly by differentiating into mature endothelial cells. In particular, the CD34^{+} hematopoietic stem cell has raised a lot of attention in this respect because of the similarities with the embryonic hemangioblast, which gives rise not only to circulating blood cell lineages but also to vascular cells.[29] Hematopoietic stem cells appear to be essential for angiogenesis in the mouse embryo[30] and, when durably engrafted in adult mice, were shown to have functional hemangioblast activity and develop into endothelial cells that participate in the neovasculature that evolved after retinal ischemia.[31]

Although bone marrow transplantation experiments have shown unequivocally that bone marrow-derived cells can also differentiate into vascular cells in situ, the frequency of this phenomenon and the identification of the cell type involved are still matter of debate.[32] Only recently have some specific surface markers for "true" EPCs emerged from detailed studies characterizing mammalian embryogenesis and angiogenesis. Flk-1/KDR is a receptor for vascular endothelial cell growth factor (VEGFR-2), which appears to be critical for embryonic endothelial cell differentiation and vasculogenesis.[33] Also, it was reported that Flk-1–positive cells, derived from differentiated embryonic stem cells, can give rise to endothelial cells and vascular smooth muscle cells in vitro and in vivo.[34]

Together with the essential role of Flk-1 in hematopoiesis,[35] these observations are consistent with the existence of a Flk-1–positive hemangioblast that serves as a common origin of endothelial cells and blood cells.[36] AC133 is a second early hematopoietic stem cell marker that is downregulated on differentiation and is therefore a marker for early EPCs. Indeed, AC133-positive cells from human peripheral blood were shown to differentiate into endothelial cells in vitro.[37] Using these stem cell markers, it has become clear that only a very small subset of circulating mononuclear cells in peripheral human blood stains (0.002%) positively for CD34, AC133, and Flk-1 simultaneously.[38] The most detailed phenotypic description of the circulating EPC (CEP) proposes the co-expression of several common endothelial and hematopoietic antigens: CD34^{+}, FGFR^{+}, CD38^{+}, VE-cadherin^{+}, c-kit^{+}, CD31^{+}, Flt-1, AC133^{+}; in addition, it represents even a subfraction of these.[39]

Are these CEP part of the inflammatory response on vascular occlusion and, if so, does contribution of these cells matter in view of the reported effects of the abundantly present monocytes on these processes? To play such a role, CEP should have the capacity to home exclusively on sites of angiogenesis. They should be able to attach to activated endothelium or extracellular matrix, to (trans)differentiate into an endothelial phenotype, and be able to proliferate. We recently demonstrated that CD34^{+} hematopoietic stem cells specifically home and migrate to angiogenic endothelium (unpublished observation). Although CD34^{+} cells probably

do not adhere to normal endothelium, they can attach to activated endothelium. Platelets may play an important modulating role in this attachment. Platelets can adhere to inflamed endothelial cells or to exposed extracellular matrix, where they express P-selectin and thus can provide an adhesive surface for CEP;[40] however, CD34$^+$ cells express the binding determinant for P-selectin (PSGL-1).[41] In particular, the issue whether endothelial progenitor cells can proliferate after homing and (trans)differentiation may be important in appreciating the in vivo relevance of these CEP as sources of paracrine factors and as sources for endothelial cells versus the effects that other inflammatory cells have on resident endothelium. Bone marrow transplantation experiments show low to very low percentages of in situ differentiation of bone marrow-derived cells into endothelial cells, making the role for CEPs as a major source of endothelial cells in the short-term perspective of these experiments less likely. However, in acute ischemic events such as myocardial infarction, the number of circulating CD34$^+$ cells was increased and a direct correlation with plasma levels of VEGF was shown.[42] Furthermore, in patients experiencing an acute vascular insult secondary to burns or coronary bypass grafting, a rapid increase (50×) in the number of CEP was noted within 6 to 12 hours after injury, which coincided with an elevation in VEGF level.[43] Clearly, increased numbers of circulating CD34$^+$ cells, in combination with efficient homing and ultimately (trans)differentiation and proliferation at the site of vascular injury, may increase the contribution of these progenitor cells relative to other inflammatory cells. This phenomenon can be used therapeutically by artificially increasing the number of circulating progenitor cells in conditions such as ischemia. VEGF, basic fibroblast growth factor, angiopoietin-1, placental growth factor, and stromal cell-derived growth factor-1 have all been shown to induce EPC mobilization and recruitment.[2,44] What most of these factors have in common is that they stimulate the Akt/PKB pathway; evidence is accumulating that the Akt/PKB pathway plays a central role in stem cell recruitment and survival.[45] Activation of this pathway may also explain some of the effect of statins on in vivo (re)endothelialization, an effect that appears, at least partly, dependent on incorporation of bone marrow-derived cells into the endothelial cell monolayer.[4,46] Because EPCs are thought to be derived from the CD34$^+$ hematopoietic stem cell, an additional method to increase circulating EPCs is to use stem cell mobilizing factors, such as stem cell factor and granulocyte-macrophage colony-stimulating factor. In experimental myocardial infarction[47,48] and the ischemic hindlimb,[49] it was found that during therapy with such cytokines, circulating EPCs were mobilized into the ischemic regions and augmented neovascularization of ischemic tissue. Recently, a stimulatory effect of erythropoietin (Epo) has also been described on EPC recruitment and angiogenesis in the mouse model of inflammation- and ischemia-induced neovascularization.[50] Also, in renal anemia patients, recombinant Epo markedly increased the number of CD34$^+$ cells in the circulation.[51] Although these observations are exciting, it should be noticed that the beneficial effects of mobilizing CEPs on ischemia were observed in otherwise healthy animals. In disease states or in
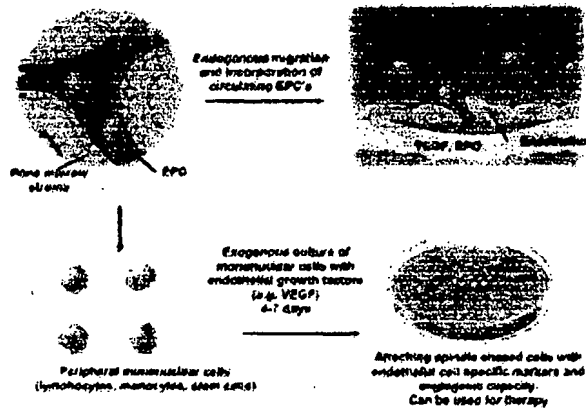
Figure 2. Endothelial progenitor cells are referred to as the endogenous CD34$^+$-derived cells that can incorporate in damaged endothelium or hypoxic tissue (upper part of the figure) as well as the ex vivo expanded and differentiated mononuclear cells, which have been shown to display endothelial-specific cell markers and angiogenic capacity (lower part of the figure).

the presence of cardiovascular risk factors, mobilization of such cells may also promote the formation of potentially harmful CD34$^+$-derived phenotypes such as macrophages or fibrocytes.[52] It thus appears that cardiovascular risk factors may shift the balance between cells that can induce repair and neovascularization toward cells that contribute to a harmful inflammatory reaction. In addition, nitric oxide availability appears to be essential for mobilization of circulating EPCs from the bone marrow stroma.[53] Strategies that allow the beneficial side of inflammation such as the endogenous capacity to form endothelial-like cells while at the same time reducing differentiation of harmful cellular phenotypes by drugs that enhance nitric oxide bioavailability or activate the Akt/PKB signaling pathway (eg, statin therapy[54,55]) may therefore prove to be even more useful than mobilizing or infusing progenitor cells.

Attaching Cells: The Other EPC

The low numbers of CD34$^+$ CEP (100 to 500 per mL blood) are in sharp contrast with the relatively large numbers of attached cells that are obtained (~100 000 from 1 mL blood) after culturing the blood mononuclear cell fraction on fibronectin or gelatin for 4 days in the presence of endothelial growth factors and that, unfortunately, often also are referred to as "EPC" (Figure 2). These spindle-shaped attaching cells (hereafter referred to as AT cells) exhibit endothelial characteristics such as the potential to take-up acetylated LDL and expression of endothelial markers such as ULEX and von Willebrand factor.[1,42] This remarkable plasticity of cells present in the AT cell cultures cannot be explained by the presence of a few co-isolated CEPs and more likely originate from a more abundant circulating mononuclear cell type, such as monocytes.[26,56-59] The concept that has developed over the years is that the number of these AT cells quantitatively reflect subpopulations within the blood mononuclear cells that have the potential to differentiate into an endothelial phenotype in vivo. Interestingly, the number of AT cells is reduced in patients with cardiovascular risk factors.[60] Recently, this reduction has been related to intermedi-

ate endpoints of cardiovascular disease, such as impaired flow-mediated dilation.[61] However, one has to realize that the AT cell cultures are an in vitro phenomenon and thus subject to methodological influences. For example, increased expression of matrix adhesion molecules such as the vitronectin receptor $\alpha_v\beta3$ in mononuclear cells (eg, by statin therapy) may yield higher numbers of AT cells in culture conditions in which vitronectin is used as an adhesive surface.[4,62] Thus, when certain adhesion receptors are altered in mononuclear cells, either disease- or therapy-related, then this will be noticed only when the mononuclear cells are cultured on the relevant adhesive protein(s). To date, ≈6 different adhesive surfaces have been used in the EPC culture assay: FN, FN plus gelatin, gelatin, VN plus gelatin, FN plus collagen, and collagen type I. To what extent these different surfaces have led to conflicting interpretations is not clear. However, we feel that the concept that the number of AT cells quantitatively reflects the number of circulating EPCs has to be carefully interpreted. Nevertheless, these AT cells appear to offer spectacular therapeutic opportunities. Intravenous infusion of these AT cells in animal models of ischemia results in homing of these cells to the ischemic tissue and augmentation of neovascularization.[5,9,61] These effects are specific, because infusion with mature endothelial cells had no such an effect.[5] Based on these observations, clinical studies have been initiated such as the TOP-CARE study, to investigate whether infusion of autologous expanded AT cells in patients with myocardial infarction reduces ischemic injury.[11]

In conclusion, evidence is accumulating that, as part of the response to hypoxia, circulating endothelial progenitor cells are mobilized from the bone marrow and subsequently differentiate into proper endothelial cells. There are also indications that such CEPs can facilitate endothelial repair and angiogenesis in vivo. It is not clear yet, however, whether CEPs are essential for these adaptive processes or what the relative contribution of CEP is compared with that of other mononuclear inflammatory cells. Moreover, there are still many uncertainties about how cardiovascular risk factors modulate CEP function. Particularly when therapeutically mobilizing CEPs, a further understanding of this issue is essential to assess the risk of transdifferentiation of CEPs to potentially pro-atherogenic inflammatory cells.[64,65]

References

1. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275:964-967.
2. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med*. 2003;9:702-712.
3. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res*. 1999;85:221-228.
4. Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T, Nishimura H, Losordo DW, Asahara T, Isner JM. Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. *Circulation*. 2002;105:3017-3024.
5. Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, Li T, Isner JM, Asahara T. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A*. 2000;97:3422-3427.
6. Schatteman GC, Hanlon HD, Jiao C, Dodds SG, Christy BA. Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. *J Clin Invest*. 2000;106:571-578.
7. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*. 2001;104:1046-1052.
8. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Amano K, Iba O, Imada T, Iwasaka T. Improvement of collateral perfusion and regional function by implantation of peripheral blood mononuclear cells into ischemic hibernating myocardium. *Arterioscler Thromb Vasc Biol*. 2002; 22:1804-1810.
9. Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, Silver M, Ma H, Kearney M, Isner JM, Asahara T. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation*. 2001;103:634-637.
10. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med*. 2001;7:430-436.
11. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation*. 2002;106:3009-3017.
12. Carmeliet P. Angiogenesis in health and disease. *Nat Med*. 2003;9: 653-660.
13. Schaper W, Scholz D. Factors regulating arteriogenesis. *Arterioscler Thromb Vasc Biol*. 2003;23:1143-1151.
14. De Keulenaer GW, Chappell DC, Ishizaka N, Nerem RM, Alexander RW, Griendling KK. Oscillatory and steady laminar shear stress differentially affect human endothelial redox state: role of a superoxide-producing NADH oxidase. *Circ Res*. 1998;82:1094-1101.
15. Topper JN, Cai J, Falb D, Gimbrone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci U S A*. 1996;93:10417-10422.
16. Hojo Y, Saito Y, Tanimoto T, Hoefen RJ, Baines CP, Yamamoto K, Haendeler J, Asmis R, Berk BC. Fluid shear stress attenuates hydrogen peroxide-induced c-Jun NH2-terminal kinase activation via a glutathione reductase-mediated mechanism. *Circ Res*. 2002;91:712-718.
17. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol*. 2000;279:L1005-L1028.
18. Baeuerle PA. IkappaB-NF-kappaB structures: at the interface of inflammation control. *Cell*. 1998;95:729-731.
19. Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol*. 1998;16:225-260.
20. Hajra L, Evans AI, Chen M, Hyduk SJ, Collins T, Cybulsky MI. The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. *Proc Natl Acad Sci U S A*. 2000;97:9052-9057.
21. Safran M, Kaelin WG, Jr. HIF hydroxylation and the mammalian oxygen-sensing pathway. *J Clin Invest*. 2003;111:779-783.
22. Chandel NS, Schumacker PT. Cellular oxygen sensing by mitochondria: old questions, new insight. *J Appl Physiol*. 2000;88:1880-1889.
23. Moncada S, Erusalimsky JD. Does nitric oxide modulate mitochondrial energy generation and apoptosis? *Nat Rev Mol Cell Biol*. 2002;3:214-220.
24. Sunderkotter C, Steinbrink K, Goebeler M, Bhardwaj R, Sorg C. Macrophages and angiogenesis. *J Leukoc Biol*. 1994;55:410-422.
25. Arras M, Ito WD, Scholz D, Winkler B, Schaper J, Schaper W. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. *J Clin Invest*. 1998;101:40-50.
26. Fernandez Pujol B, Lucibello FC, Gehling UM, Lindemann K, Weidner N, Zuzarte ML, Adamkiewicz J, Elsasser HP, Muller R, Havemann K. Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation*. 2000;65:287-300.

27. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* 2001;107:1395-1402.

28. Zhao Y, Glesne D, Huberman E. A human peripheral blood monocyte-derived subset acts as pluripotent stem cells. *Proc Natl Acad Sci U S A.* 2003;100:2426-2431.

29. Eichmann A, Corbel C, Nataf V, Vaigot P, Breant C, Le Douarin NM. Ligand-dependent development of the endothelial and hemopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. *Proc Natl Acad Sci U S A.* 1997;94: 5141-5146.

30. Takakura N, Watanabe T, Suenobu S, Yamada Y, Noda T, Ito Y, Satake M, Suda T. A role for hematopoietic stem cells in promoting angiogenesis. *Cell.* 2000;102:199-209.

31. Grant MB, May WS, Caballero S, Brown GA, Guthrie SM, Mames RN, Byrne BJ, Vaught T, Spoerri PE, Peck AB, Scott EW. Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization. *Nat Med.* 2002;8:607-612.

32. Voswinckel R, Ziegelhoeffer T, Heil M, Kostin S, Breier G, Mehling T, Haberberger R, Clauss M, Gaumann A, Schaper W, Seeger W. Circulating vascular progenitor cells do not contribute to compensatory lung growth. *Circ Res.* 2003;93:372-379.

33. Shalaby F, Ho J, Stanford WL, Fischer KD, Schuh AC, Schwartz L, Bernstein A, Rossant J. A requirement for Flk1 in primitive and definitive hematopoiesis and vasculogenesis. *Cell.* 1997;89:981-990.

34. Yamashita J, Itoh H, Hirashima M, Ogawa M, Nishikawa S, Yurugi T, Naito M, Nakao K, Nishikawa S. Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature.* 2000;408: 92-96.

35. Ziegler BL, Valtieri M, Porada GA, De Maria R, Muller R, Masella B, Gabbianelli M, Casella I, Pelosi E, Bock T, Zanjani ED, Peschle C. KDR receptor: a key marker defining hematopoietic stem cells. *Science.* 1999; 285:1553-1558.

36. Choi K, Kennedy M, Kazarov A, Papadimitriou JC, Keller G. A common precursor for hematopoietic and endothelial cells. *Development.* 1998; 125:725-732.

37. Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, Schuch G, Schafhausen P, Mende T, Kilic N, Kluge K, Schafer B, Hossfeld DK, Fiedler W. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood.* 2000;95:3106-3112.

38. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, Rafii S. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood.* 2000;95:952-958.

39. Burger PE, Coetzee S, McKeehan WL, Kan M, Cook P, Fan Y, Suda T, Hebbel RP, Novitzky N, Muller WA, Wilson EL. Fibroblast growth factor receptor-1 is expressed by endothelial progenitor cells. *Blood.* 2002;100:3527-3535.

40. Janowska-Wieczorek A, Majka M, Kijowski J, Baj-Krzyworzeka M, Reca R, Turner AR, Ratajczak J, Emerson SG, Kowalska MA, Ratajczak MZ. Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. *Blood.* 2001;98: 3143-3149.

41. Hidalgo A, Weiss LA, Frenette PS. Functional selectin ligands mediating human CD34(+) cell interactions with bone marrow endothelium are enhanced postnatally. *J Clin Invest.* 2002;110:559-569.

42. Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation.* 2001;103: 2776-2779.

43. Gill M, Dias S, Hattori K, Rivera ML, Hicklin D, Witte L, Girardi L, Yurt R, Himel H, Rafii S. Vascular trauma induces rapid but transient mobilization of VEGFR2(+)AC133(+) endothelial precursor cells. *Circ Res.* 2001;88:167-174.

44. Szmitko PE, Pedak PW, Weisel RD, Stewart DJ, Kutryk MJ, Verma S. Endothelial progenitor cells: new hope for a broken heart. *Circulation.* 2003;107:3093-3100.

45. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med.* 2003;9:1195-1201.

46. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rutten H, Fichtlscherer S, Martin H, Zeiher AM. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest.* 2001;108:391-397.

47. Norol F, Merlet P, Isnard R, Sebillon P, Bonnet N, Cailliot C, Carrion C, Ribeiro M, Charlotte F, Pradeau P, Mayol JF, Peinnequin A, Drouet M, Safsafi K, Vernant JP, Herodin F. Influence of mobilized stem cells on myocardial infarct repair in a nonhuman primate model. *Blood.* 2003; 102:4361-4368.

48. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A.* 2001;98:10344-10349.

49. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med.* 1999;5:434-438.

50. Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, Mildner-Rihm C, Martin H, Zeiher AM, Dimmeler S. Erythropoietin is a potent physiological stimulus for endothelial progenitor cell mobilization. *Blood.* 2003;102:1340-1346.

51. Bahlmann FH, DeGroot K, Duckert T, Niemczyk E, Bahlmann E, Boehm SM, Haller H, Fliser D. Endothelial progenitor cell proliferation and differentiation is regulated by erythropoietin Rapid Communication. *Kidney Int.* 2003;64:1648-1652.

52. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol.* 2001;166:7556-7562.

53. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med.* 2003;9:1370-1376.

54. Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, Dimmeler S. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation.* 2001; 103:2885-2890.

55. Llevadot J, Murasawa S, Kureishi Y, Uchida S, Masuda H, Kawamoto A, Walsh K, Isner JM, Asahara T. HMG-CoA reductase inhibitor mobilizes bone marrow-derived endothelial progenitor cells. *J Clin Invest.* 2001; 108:399-405.

56. Harraz M, Jiao C, Hanlon HD, Hartley RS, Schatteman GC. Cd34(-) blood-derived human endothelial cell progenitors. *Stem Cells.* 2001;19: 304-312.

57. Schmeisser A, Garlichs CD, Zhang H, Eskafi S, Graffy C, Ludwig J, Strasser RH, Daniel WG. Monocytes coexpress endothelial and macrophagocytic lineage markers and form cord-like structures in Matrigel under angiogenic conditions. *Cardiovasc Res.* 2001;49:671-680.

58. Rehman J, Li J, Orschell CM, March KL. Peripheral blood 'endothelial progenitor cells' are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation.* 2003;107:1164-1169.

59. Rookmaaker MB, Vergeer M, van Zonneveld AJ, Rabelink TJ, Verhaar MC. Endothelial progenitor cells: mainly derived from the monocyte/macrophage containing CD34- but also partly derived from the hematopoietic stem cell containing CD34+ mononuclear population. *Circulation.* 2003;108:150e.

60. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res.* 2001;89:E1–E7.

61. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003;348:593-600.

62. Kalka C, Masuda H, Takahashi T, Gordon R, Tepper O, Gravereaux E, Pieczek A, Iwaguro H, Hayashi SI, Isner JM, Asahara T. Vascular endothelial growth factor(165) gene transfer augments circulating endothelial progenitor cells in human subjects. *Circ Res.* 2000;86:1198-1202.

63. Aicher A, Brenner W, Zuhayra M, Badorff C, Massoudi S, Assmus B, Eckey T, Henze E, Zeiher AM, Dimmeler S. Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. *Circulation.* 2003;107:2134-2139.

64. Caplice NM, Bunch TJ, Stalboerger PG, Wang S, Simper D, Miller DV, Russell SJ, Litzow MR, Edwards WD. Smooth muscle cells in human coronary atherosclerosis can originate from cells administered at marrow transplantation. *Proc Natl Acad Sci U S A.* 2003;100:4754-4759.

65. Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, Hirai H, Makuuchi M, Hirata Y, Nagai R. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat Med.* 2002;8:403-409.